Targeting purinergic receptors to suppress the cytokine storm induced by SARS-CoV-2 infection in pulmonary tissue

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ABSTRACT

The etiological agent of coronavirus disease (COVID-19) is the new member of the Coronaviridae family, a severe acute respiratory syndrome coronavirus 2 virus (SARS-CoV-2), responsible for the pandemic that is plaguing the world. The single-stranded RNA virus is capable of infecting the respiratory tract, by binding the spike (S) protein on its viral surface to receptors for the angiotensin II-converting enzyme (ACE2), highly expressed in the pulmonary tissue, enabling the interaction of the virus with alveolar epithelial cells promoting endocytosis and replication of viral material. The infection triggers the activation of the immune system, increased purinergic signaling, and the release of cytokines as a defense mechanism, but the response can become exaggerated and prompt the so-called "cytokine storm", developing cases such as severe acute respiratory syndrome (SARS). This is characterized by fever, cough, and difficulty breathing, which can progress to pneumonia, failure of different organs and death. Thus, the present review aims to compile and correlate the mechanisms involved between the immune and purinergic systems with COVID-19, since the modulation of purinergic receptors, such as A2A, A2B, and P2X7 expressed by immune cells, seems to be effective as a promising therapy, to reduce the severity of the disease, as well as aid in the treatment of acute lung diseases and other cases of generalized inflammation.

1. Introduction

In the past twenty years, several viral epidemics have been recorded. Among them, the severe acute respiratory syndrome (SARS-CoV) in 2002 and 2003, the H1N1 flu in 2009 and the Middle East respiratory syndrome (MERS-CoV) that was identified in Saudi Arabia in 2012. In 2020, with defined etiology by the Chinese Centers for Disease Control and Prevention (CDC), a new virus of the Coronaviridae family was able to initiate a pandemic, with the first cases reported in early December 2019 in the Hubei province, Wuhan, China [1].

Starting as an epidemic of cases of respiratory infections, on February 11, 2020, the Director-General of the World Health Organization (WHO), Dr. Tedros Adhanom Ghebreyesus, announced the name of the disease caused by this new CoV, COVID- 19, and in February, the International Virus Classification Committee also named the SARS-CoV-2 virus [1]. In this context, since 2003, health organizations have coordinated guidelines and sought diagnoses, as well as the clinical spectrum of the disease and prevention strategies, since it is known that CoVs are considered the main pathogens of respiratory disease outbreaks.

As it is an acute infectious respiratory disease, transmission occurs mainly through the respiratory tract, through coughing, sneezing and droplets of saliva from infected people and contact with contaminated objects. As for clinical symptoms, in milder cases, fever, dry cough, fatigue, gastrointestinal infections and dyspnea stand out, while in critical situations hypoxia and pneumonia can occur [2,3].

In this perspective, the pathogenic mechanism of SARS-CoV-2 occurs similarly to that studied in SARS-CoV, as it affects the respiratory tract and signals the same receptor and enzyme [3]. Research has shown that the virus has a greater affinity for cells in the respiratory tract and uses the S protein present in its lipid layer to attach itself to cells in the respiratory system. The virus uses a cell membrane protease (TMPRSS2) - which cleaves the S protein - and a receptor for the ACE2 to bind to the cell and replicate its genetic material.

During the course of infection, the host cells undergo the process of viral replication until cellular lysis, responsible for releasing 10,000 to 100,000 copies of viruses, which can infect new cells and the organism as a whole. A recent study has shown that an individual may be more susceptible if, in the initial stage of viral infection, the viral receptor presents greater binding affinity between the host cell receptors, in addition to the number of receptors expressed on the cells in each human being this susceptibility also varies [4,5].

These and other chemical and genetic associations are reasons why some people may have the severe form of the disease and in others the symptoms are mild [4,5], associated with a family history of lung diseases, other comorbidities such as diabetes and hypertension and progression of infection and inflammation.

Regarding the COVID-19 immunization program, science advances in relation to vaccine research and population immunization in Brazil and worldwide. From this perspective, different vaccines were considered safe to be administered in the population, such as Pfizer-BioNTech, Moderna and AstraZeneca. In addition, in November 2020, the Gamaleya Research Institute announced an interim analysis based on 18,794 volunteers who received the first and second doses of the Sputnik V vaccine. As a result, an efficacy of 91.4% was observed seven days after the second dose, followed by a protective efficacy greater than 95% after two weeks [6]. In conjunction with the increased coverage of vaccination, inflammatory reactions tend to be greater when compared to flu vaccines [7]. However, no safety concerns appear to be related to the administration of Pfizer - BioNTech, Moderna, Oxford / AstraZeneca and Gamalaya Res. After administration of the vaccine, the frequency of inflammatory reactions is greater than that commonly seen with flu vaccines. It is noteworthy that, according to studies, AstraZeneca, the candidate vaccine ChAdOx1 induced a similar immune response in all age groups, although it was less reactogenic in older adults than in younger adults [8].

Coronavirus infection signals the immune system, which is a complex network of organs, tissues, and cells that act in the protection of our organism against toxic substances and invading microorganisms, such as fungi, viruses, and bacteria. Thus, with the entry of the virus, different antibodies and defense cells are recruited to fight the infection, returning to the baseline situation with the destruction of the pathogen. However, in situations in which the body lacks effective defenses, the pro-inflammatory immune pathways can become uncontrolled and generate an inflammatory process that is harmful to the body [9].

This exacerbated response can become generalized and capable of compromising the entire organism, since cells of the immune system are found in all organs, including blood and lymphatic vessels. The immune response can be divided into innate - considered nonspecific and offering protection since birth - and acquired, considered specific with the development after the contact with several microorganisms. Thus, the immune system must be studied to understand COVID-19 and how the uncontrolled organization is capable of promoting a worse prognosis [9].

When the virus infects the host cell, the innate immune response is immediately activated and the natural killer (NK) cells, along with macrophages, are directed to the tissue site to contain the virus's progress. In this scenario, some endocytosis has already occurred, leading to the infection of the local cells, and the function of the NK cells is to induce apoptosis of the infected cells, associated with a phagocytic action of macrophages and release of cytokines. In mild situations, only the innate immune response is sufficient to eliminate the pathogen and restore homeostasis to the body [10].

However, in the most severe cases of COVID-19 disease - about 20% according to WHO data - individuals develop more critical symptoms, such as difficulty in breathing, severe pneumonia, hypoxia, organ failure, and risk of death; in these events only the innate response is not enough to eliminate the virus, requiring the recruitment of lymphocytes and the production of antibodies, among other components of the immune system [10]. The initial release of cytokines and the recruitment of immune cells, such as CD4 + lymphocytes, may lack control and trigger the so-called "cytokine storm". This process generates an increase in the necrotic process and promotes the inflammatory condition in the pulmonary alveoli, causing edema, which brings fluids to the interior of the alveoli and also to the blood capillaries next to them, generating the clinical picture of pneumonia [10].

One of the main causes that lead to the worsening of COVID-19 is a cytokine storm, one of which is responsible for causing Acute Respiratory Distress Syndrome (ARDS) and multiple organ failure, occurring in death in a short period of time [11]. The cytokine storm mechanism during a viral infection is directly linked to the exacerbation of the immune response and an uncontrolled release of inflammatory markers [12]. Even in the early stages of infection, as epithelial cells trigger a series of signaling processes with the slow release of cytokines and chemokines, dendritic cells and macrophages, followed by the secretion of interferons from antiviral factors (IFNs) and high levels of pro-inflammatory cytokines (interleukin (IL) -1β, IL-6, IL-7; tumor necrosis factor (TNF)); chemokines (chemokine ligand with CC motif (CCL)) -2, CCL-3 and CCL-5); granulocyte colony-stimulating factor (G-CSF) [13].

With the increase in these levels, there is then a greater activation and signaling to the cells of the immune system, mainly T cells, monocytes and macrophages, which make a positive feedback, through interferon Gamma, to produce more cytokines [14]. Therefore, an excessive response occurs, with infiltration of monocytes, macrophages and T cells, generating edema, making oxygen capture difficult, hindering gas exchange and a drastic drop in saturation. Furthermore, the cytokine storm can also affect other organs, cause heart, kidney, liver problems, among others [11]

In this generalized inflammatory situation, the organism is under stress due to the increase in the production of proinflammatory mediators associated with elevation of extracellular levels of adenosine triphosphate (ATP) and other nucleotides. The overflow of adenine

nucleotides occur through cell lysis and these signaling molecules trigger a hyper stimulation of purinergic receptors. It is known that this event mediated by ATP, although it is a physiological phenomenon, when unbalanced culminates in a cytokine storm generating numerous pathologies. A healthy individual has a practically insignificant concentration of ATP in the extracellular environment (at the nanomolar range), however, in the intracellular environment, this amount is quite significant (reaching several millimolar), as it is a molecule that provides energy to the cell [15].

Thus, it is understood that the purinergic signaling is present in all aspects of immunity and inflammation and, increasingly, studies show that extracellular ATP, together with its adenosine (Ado) metabolite, are chief mediators of inflammatory responses. This occurs since most immune cells express P2 and P1 receptors, which are sensitive to the ATP and Ado molecules, respectively. In addition, depending on the amount of ATP in the extracellular environment, this molecule can act as an immunostimulant or immunodepressor, on the other hand, the concentration of Ado has anti-inflammatory actions and is considered a potent immunosuppressive molecule [16].

Along with the cytokine storm, thrombotic processes such as thrombosis and disseminated intravascular coagulation (DIC) are highly associated with the severity of COVID-19 and worse prognosis [17]. Knowledge about the incidence of thrombotic processes in patients affected with COVID-19 is very important for decision making regarding thrombus prophylaxis, especially when the patient is hospitalized in the intensive care unit (ICU) in which the thrombotic risk is even greater [18].

A fibrotic coagulation abnormality with a significant increase in D-dimers was reported in several studies in patients who had a greater severity of the pathology. D-dimers are produced in the blood through the breakdown of fibrin. Thus, thrombi are formed from coagulation activation and are decomposed through fibrinolytic activation. However, evaluating the D-dimer dosage in isolation is not recommended to assess prognosis and is not directly correlated with the severity of the pathological condition, given that the coagulation condition and fibrinolytic pathologies can fluctuate in a short time. From this perspective, regular exams are important and follow-up is necessary every 2–3 days [19,20]. Added to this, other clinical conditions, such as in the fibrinolytic-type ICD caused by sepsis, the level of D-dimer also remains low [21].

Both arterial and venous thromboses were reported in COVID-19, but venous thromboembolism was shown to have a high incidence and was associated with more severe cases. In addition, pulmonary thrombosis was also shown to be more frequent than pulmonary embolism. It is worth noting that the pathophysiology of DIC in patients affected with COVID-19 is different from that of septic DIC, and both pathologies (thrombotic and hemorrhagic) must be observed during the patient's assessment and treatment [19]. COVID-19 thrombosis includes macro and microthrombosis, the diagnosis of which is dependent on coagulation and fibrinolysis markers. Furthermore, it is important to emphasize the difference between diagnosis between micro and macrothrombosis. While macro can be diagnosed by contrast-enhanced computed tomography (CT), microtrobosis is at the microscopic level, and shows thrombi in the small arteries and small veins of the lung, which contained fibrin and platelet components. Therefore, to differentiate these findings, an autopsy must be performed. The most used anticoagulant therapy to date for COVID-19 is heparin, due to its anticoagulant, antiviral and anti-inflammatory effects [19].

Based on this brief context, this study will highlight the therapeutic potential of purinergic signaling in SARS-CoV-2 infection, especially in the respiratory tract, since the increase in extracellular nucleotide levels promoted by the cytokine storm is able to further trigger a systemic inflammatory condition and affect several organs. One of the forms of this increase is the lysis of pulmonary endothelial cells such as type II pneumocytes and the extravasation of intracellular ATP to the external environment, increasing inflammation; however, studies show that Ado is capable of reversing this condition by exerting local and systemic anti-inflammatory properties. These actions can occur through the signaling of P1 receptors or can be related to the inhibition of P2 receptors [22]. Thus, the study of these adenine signaling molecules is shown to be necessary, since the modulation of their levels especially in the extracellular milieu seems to be able to reduce and even control the cytokine storm, minimizing the damage caused by the SARS-CoV-2 infection and potentially reducing its lethality.

2. Purinergic system

The purinergic system is defined as a set of components capable of generating a cellular intercommunication network [23]. Composed of signaling molecules, regulatory enzymes, and specific receptors, this organization is capable of modulating several basal pathways of the organism. Currently it has been widely studied for its therapeutic potential and modulation of physiological processes, such as apoptosis, thromboregulation, cell proliferation, platelet aggregation, endothelial vasodilation, and pain [24–27], as well as neurotransmission and neuroprotection [28]. In addition, promising studies [29–31] also report an association of the purinergic system with inflammatory processes and immune responses, as well as in immunological diseases [32,33], neurodegeneration [34-36], psychiatric illnesses [37], cancer [38], diabetes and hypertension [39].

Composed by nucleoside and nucleotides are signaling biomolecules that have functional activities in the extracellular environment and modulate several biological responses [40]. Biochemical interactions of the protein structures – enzymes and receptors – with purines - heterocyclic aromatic molecules [41], including ATP, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) which constitute the nucleotides, whereas Ado constitutes the nucleoside [42]. These are regulated by specific enzymes, ectoenzymes, which are divided into adenosine deaminase (ADA), responsible for deaminating Ado to inosine, and ectonucleotidases such as E-NPP (ectonucleotide pyrophosphatase/ phosphodiesterase), E-NTPDases (ectonucleoside triphosphate diphosphohydrolase), ecto-5′-nucleotidase (E-5′-NT), and alkaline phosphatases (ALP) that regulate nucleotides levels.

In this view, the receptors can be initially classified into P1 and P2 groups, according to their structural characteristics and biochemical functionalities. The first class, P1 receptors, are closely related to the nucleoside Ado and coupled to the G protein being subdivided into A1, A2A, A2B, and A3. The second group, P2 receptors, are sensitized by mono-, di-, and triphosphate nucleotides, such as AMP, ADP, and ATP, respectively, being subdivided into a) P2X (1–7) subtype of receptors, when activated, result in the opening of pores in the cell membrane and allow the passage of Na^+, K^+, and Ca^{2+} cations, and b) P2Y (1, 2, 4, 6, 11–14) metabotropic G-protein coupled receptors [40].

Considered an important nucleoside, Ado is formed by the binding of an adenine to a ribose, which can be formed by the hydrolysis of ATP, by the cleavage of S-adenosyl-homocysteine [43] or by the action of the 5′-nucleotidase enzyme in degrading AMP [44]. Regardless of the origin, Ado can be present in the intra and extracellular environment, acting fundamentally in the regulation of homeostasis and as a neuromodulator, as mentioned by Cunha [45], and may be involved in the regulation of sleep [46,47], cognition, and memory [48-50].

In many pathological processes, Ado is able to limit damage and serve as an anti-inflammatory molecule by regulating the release of excitatory neurotransmitters, acting as a neuroprotector [43]. Ado have other functions include the involvement in the synthesis of nucleic acids, modulation of the cellular metabolic state, and metabolism of amino acids [51,52]. Furthermore, when acting on the central nervous system (CNS), it has a direct relationship with proliferation, necrosis, apoptosis, and signaling of cellular damage. As a potential immunosuppressant, Ado modulates anti-inflammatory actions, inhibits platelet aggregation, stimulates cell migration, increases vasodilatation in order to endogenously regulate innate immunity and inflammation-related tissue

defenses, thus protecting the heart [16,26,53].

Acting as a signaling molecule in the purinergic system, ATP is involved in the regulation of several pathophysiological processes in the extracellular environment. Present in all cells, ATP remains stored in vesicles at the synaptic endings and it is released after neuronal depolarization acting on the postsynaptic membrane at specific receptors, called purinoreceptors [54-56]. In addition, it can be released together with several neurotransmitters, such as acetylcholine (ACh), glutamate, norepinephrine, serotonin, and γ-amino butyric acid (GABA) [30,57-62].

The signaling initiated by the release of ATP occurs by passage through the membrane through exocytosis [63] or through channels permeable to ATP [64]. One of the families of these channels is pannexin and consists of three members in humans: PANX1, 2 and 3 [65]. Thus, PANXs, widely expressed throughout the body, are large-pore non-selective channels that share a 4-transmembrane domain arrangement with connexins, inexins, volume-regulated anion channels (VRACs) and calcium homeostasis modulators (CALHMs) [66,67]. Thus, it plays crucial roles in physiological processes such as blood pressure regulation, glucose uptake, inflammation and cell death [68].

PANX1 has been found to be activated under various physiological and/or pathological conditions. Under normal cellular conditions, PANX1 is self-inhibited by its C-terminal tail (CTT) [69]. However, the channel can still be activated by membrane depolarization, extracellular potassium, intracellular calcium, tyrosine phosphorylation, and mechanical stretching by unknown mechanisms [70]. Cleavage of CTT during apoptosis or experimentally by caspase 3 or 7 results in channel activation and ATP release [71]. Thus, although the release of ATP to the extracellular environment is natural, lack of control in pathological scenarios is capable of exacerbating inflammation.

In cases of hypoxia or injury, the ATP released in the presynaptic terminals can reach the postsynaptic end and may trigger physiological response mechanisms associated to cell damage and demise [16,72]. In this perspective, it is necessary to highlight that the actions triggered by this nucleotide can vary according to the physiological concentration, the place of action, and the concentration of receptors of this molecule. Therefore, it can exert different responses and for this reason both nucleotides and nucleoside can modulate pro- and anti-inflammatory activities [28].

In the situation of cell lysis, the intracellular content is released - the internal concentration of ATP in this environment can vary from 5 to 10 mM -, and with its concentration increased in the intracellular milieu, the inflammatory process is signaled. From this point of view, in pathophysiological situations there may be an increase in ATP levels and overexpression of the respective purinoreceptors [73]. Thus, the control of the extracellular concentration of this nucleotide is done through the activity of ectonucleotidases, catalyzing ATP to Ado, since its passage through biological membranes by diffusion or active transport is not possible [74-76].

Defined as regulators of the extracellular concentration of nucleotides and nucleosides, purinergic ectoenzymes become relevant in many physiological processes, given their hydrolytic power [25,27,72,77]. Anchored to the cell membrane, these enzymes have the active site facing the extracellular medium, and also may be soluble in the interstitial medium. This group of proteins is comprised by the families of E-NTPDase, E-NPP, E-5′-NT, and ALPs [25,72,77,78]. These ectonucleotidases are responsible for the hydrolysis of nucleotides to its respective nucleoside, constituting a highly efficient enzymatic cascade, being in charge of regulating the concentration and the time that these signaling molecules remain in the extracellular environment, and consequently stimulating their receptors [79].

As for E-NTPDases, the eight members of this family of enzymes are responsible for the hydrolysis of both ATP and ADP into AMP, depending on their enzymatic specificities and requirements of Ca^{2+} and Mg^{2+} ions [72,80]. These enzymes are expressed in several tissues and cell types, including the heart, lungs, placenta, thymus, kidneys, reproductive system, brain, liver, skeletal muscles, and human lymphocytes [72]. Among other functions, E-NTPDases may influence the functionality of lymphocytes, including the generation of signals to promote cellular interaction, recognition of antigens, and activation of T cytotoxic cells [72].

The ectonucleoside diphosphohydrolase-1 triphosphate, also called CD39, represents one of the most expressed ectoenzymes on the surface of regulatory T cells (Treg) [72,81]. CD73, also known as E-5′-NT, has a signaling function during cell–cell and cell-matrix adhesion process and is generally related to the activation and maturation of lymphocytes, and resistance of tumor cell lines to chemotherapeutic agents [82]. In addition, the action of NTPDases enzymes are highlighted in different diseases, as they can act in neurotransmission and modulate the levels of nucleosides and nucleosides, such as 5′-nucleotidase, which increases the level of Ado and reduces the levels of ATP in the extracellular medium [26,72,83].

ADA is responsible for promoting the hydrolytic deamination of Ado in inosine. The product of the reaction represents an inactive metabolite and its deficiency is closely associated with an abnormal extracellular concentration of Ado, contributing to pathological situations [25]. In addition, it has two isoforms, ADA1 and ADA2, widely distributed in animal tissues, the first being more expressed and concentrated in serum when compared to the second. Both enzymes also function in the thymus, in peripheral lymphocytes, and in lymphoid tissues promoting the differentiation and proliferation of lymphocytes [24,57].

Thus, in the pulmonary environment, different studies demonstrate the presence of ectonucleotidase activities in human airway epithelia [84]. Thus, experiments demonstrated, in human bronchial epithelial cells, a dephosphorylation of ATP in the mucosa to ADP, AMP and adenosine. Other studies indicate an increase in ATP metabolism in the alveolar region when compared to cultures with nasal, bronchial and bronchiolar epithelial cells [84]. In addition, NTPDase 1, 2 and 3 mRNA expression was observed in total lung RNA and in bronchial epithelial cell culture [85].

Widely distributed in the human body, purinoreceptors can be found in neural tissues, such as neurons and glial cells, as well as non-neural tissues such as epithelial cells, placenta, and platelets, for example [30]. Purinergic receptors comprise a set of proteins expressed at the plasma membrane having cellular functions related to apoptosis, inflammatory processes through cytokine secretion, and vascular reactivity through the binding of specific signaling molecules. Currently, following the definitions by Abbracchio and Burnstock [86], these receptors are divided into two classes, according to the sensitivity to the respective nucleotide or nucleoside and according to the basis of the mechanism of action, pharmacological, and molecular cloning [87,88].

Receptors sensitized to Ado, classified as P1, are coupled to protein G and subdivided into A1, A2A, A2B and A3, and act by signaling fundamental intracellular pathways for cellular functioning [54]. As transmembrane receptors, P1 are metabotropic receptors that can capture extracellular signals, whose endogenous agonist is Ado and act on cellular transduction pathways [81,89].

Regarding their localization, A1 receptors are present in greater concentration in the CNS, at the cellular level they are mainly expressed in the axon and presynaptic and postsynaptic regions [90]; besides the spinal cord, brainstem, thalamus, cerebral cortex, hippocampus, and cerebellum are also structures in which these receptors are found [91]. As for its applicability, the A1 receptors have been implicated in the treatment of supraventricular tachycardia [92]. A2A receptors are also present in the CNS in areas of the brain, including the limbic system and the cortex [90], and widely distributed in peripheral tissues [93]. At the cellular level, they are concentrated in the presynaptic (hippocampus) and postsynaptic (striated) regions, centrally; they are mainly present in the olfactory tubercle, nucleus accumbens (NAc), and in the striatum. In addition, these receptors are associated with the decrease in the activation of inflammatory cells from different sites and may act in an anti-inflammatory manner on neutrophils [94], while A2A receptor

antagonists may have benefits in Parkinson's disease [95].

In contrast, A2B type of receptors have low expression in the CNS and are usually found in the pituitary, lung, and large intestine [91], and can contribute to tissue adaptation in inflammatory responses, hypoxia, and ischemia [96-98]. Finally, A3 receptors, due to the lack of ligands, are poorly studied [99] and have low expression in the CNS, however, they can be found moderately expressed in the cerebellum and hippocampus [91]. In addition to acting in the production of aqueous humor in the eye [91], their antagonism appears to be effective in the treatment of dry eye [100].

P2 types of receptors have a preference for di- and triphosphate nucleotides, such as ATP and ADP, being subdivided into P2X and P2Y types of receptors. P2X receptors are widely distributed in glial cells, neurons, and smooth muscle; they are linked to ion channels that, when activated, result in the opening of pores in the cell membrane leading to the passage of Na^+, K^+, and Ca^{2+} ions [101-106]. P2Y are metabotropic receptors coupled to a G protein [107], and distributed in several tissues and systems, such as cardiac, nervous and vascular [15,78,108,109]. Additionally, P2X ionotropic receptors are further classified into seven subtypes from P2X1-7; on the other hand, P2Y metabotropic receptors can be further categorized into eight subtypes of receptors, namely $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ [107].

In the pulmonary environment, most lung cells express distal purinergic receptors. Among them, type I and II alveolar epithelial cells, pulmonary endothelial cells, bronchial epithelial cells, as well as immune cells present in this environment such as alveolar macrophages, neutrophils, eosinophils, lymphocytes, dendritic cells [110]. Furthermore, these purinoreceptors are expressed in many blood cell types and cells of the immune system, such as erythrocytes, mast cells, T and B lymphocytes, platelets, thrombocytes, macrophages, neutrophils, dendritic cells, and NK cells [16,111,112]. Concerning their expression and functionalities, P2Y and P2X receptors modulate key responses in immune and inflammatory cells, such as cytokine release and chemotaxis. From this perspective, it is noteworthy why selective agonists and antagonists of P2X or P2Y subtypes of receptors have therapeutic potential in the pharmaceutical industry [89,112].

Based on this initial panorama and taking into consideration the close relationship between the purinergic and immune systems, especially due to nearly ubiquitous expression of purinergic proteins on immune cells and signaling functions of ATP and Ado during inflammatory responses, it becomes essential to consider, besides the potential capability of the immune system in viral infection, but also the modulation of purinergic receptors during the course of COVID-19. Some considerations on this regard will be highlighted in the following sections.

3. Immune system and inflammatory responses in severe acute respiratory syndromes

3.1. Immune mechanisms: General outlines

In viral infections, components of the immune system, such as type I interferon (IFN-I), induce an immune signaling in order to rapidly promote viral attack, thus protecting the organism from the pathogen and subsequently returning to the situation of general homeostasis [113]. The defense capacity is based on the set of organs, tissues, cells and molecules that have the ability to recognize molecular structures that are foreign to the organism. Thus, both antigens that enter the organism, as well as the body's own structures that cause their own harm, generate stimuli that promote responses from the immune system, resulting in the inactivation or destruction of the foreign or unknown body [114].

The immune system is divided into two segments: the innate or nonspecific immune system and the acquired or specific immune system, the second is acquired throughout life, after the exposure and contact with different microorganisms [9]. The innate system recognizes viral infections using pattern recognition receptors (PRRs) that bind to

pathogen-associated molecular patterns (PAMPs). PRRs mainly include the toll-like receptor (TLR), the RIG-I type receptor (RLR), the NOD type receptor (NLR), the C-type lectin receptors (CLRs) and the free molecule receptors in the cytoplasm. Thus, it is possible to recognize PAMPs, by means of lipids, lipoproteins, proteins, and nucleic acids of fungal, viral and bacterial origin, by TLRs expressed in cell membranes, endosomes, lysosomes, endocytolysomes, among other cell sites of the human organism [113,114].

Thus, each TLRs can provide different biological responses through subsequent activation of varied adapter proteins, therefore, in the event of a viral infection, the immune cascade begins with the immune defense, which is rapidly initiated [115]. The inflammatory process becomes a natural response to harmful stimuli, as a biological option to circumvent the infection; however, this pathway causes tissue damage that, in mild inflammatory processes, is quickly repaired, returning to homeostasis [116].

However, when this inflammatory response becomes uncontrolled or exaggerated, there is a greater recruitment of immune cells, proinflammatory cytokines, and damage to the surrounding tissue. Thus, vascular and cellular responses are observed, including the activation of components of the purinergic system, which lead to the characteristic signs of inflammation, such as heat, redness, pain, edema, also promoting loss of tissue and organ function in severe cases [9,116,117].

The events involved in the inflammatory process tend to be resolved in cases of acute inflammation, in which the main cells involved are neutrophils and macrophages. However, if the acute inflammation is not controlled or if the harmful stimuli persist, its permanence leads to chronic inflammation, suffering from infiltration of mononuclear cells such as monocytes, macrophages, and lymphocytes, in addition to signs of angiogenesis and fibrosis, which will consequently result in chronic inflammatory diseases [9,115].

In this context, the acute or chronic inflammatory processes, resulting from tissue damage, are observed in several organic systems. In the case of a structural loss of the plasma membrane due, for example, cell lysis, an activation of purinergic receptors in the extracellular environment can be triggered by ATP, ADP, AMP, and ADO. These signaling molecules are released by all cells, including lymphocytes and platelets, in response to cellular stimulation or damage caused by the action of pathogens [28,117]. Thus, they can be studied as markers of infection and inflammation itself.

Due to the vast exposure to pathogens and antigens present in the environment, the lungs may suffer from several inflammatory diseases. Its defense system consists of an epithelial barrier and the recruitment of immune system cells, such as neutrophils, eosinophils, and macrophages. Acute inflammation and tissue damage lead to changes in gas exchange and, in cases of chronic inflammation, some diseases may arise, such as Chronic Obstructive Pulmonary Disease (COPD), asthma, among others [116,118]

The inflammatory response in the lungs can also be found in different syndromes, such as the ARDS, which is a severe syndrome characterized as a type of respiratory failure resulting from injuries in the alveolar epithelium and capillary endothelium through direct mechanisms, such as aspiration of gastric contents, pneumonia, injury by inhalation, and pulmonary contusion, or indirect mechanisms, such as sepsis, trauma, and pancreatitis. The lesion in the alveolar-capillary membrane contributes to the reduction of pulmonary plasticity, interstitial and alveolar edema, causing changes in gas exchange, generating hypoxemia, which can cause failure of multiple organs and tissues [119,120].

3.2. Severe acute respiratory syndrome (SARS)

Severe Acute Respiratory Syndrome (SARS) is characterized as an infectious disease, caused by several etiologic agents such as influenza A (H1N1) virus, dengue fever, respiratory syncytial virus, adenovirus, hantavirus, and coronavirus, in addition to bacteria such as pneumococcus, *Legionella sp.*, Leptospirosis. Thus, patients with SARS are

considered to have had Flu Syndrome, defined as a clinical picture of fever, accompanied by cough, sore throat or difficulty breathing, in addition to headache, myalgia or arthralgia. In addition, they may be accompanied by gastrointestinal manifestations or other symptoms, and should be monitored, as conditions such as tachypnea, SpO2 saturation < 95% in room air, signs of respiratory distress and hypotension are often present with increasing severity. Thus, acute respiratory failure occurs, with laboratory changes in the blood count (leukocytosis, leukopenia or neutrophilia) [9,121].

In Guangdong, China, the disease was described as atypical pneumonia, the virus being called SARS-CoV, belonging to the Coronaviridae family, the etiologic agent. The lack of previous seroepidemiological data led the study to findings that suggested SARS-CoV zootropism since it was not an epidemic in humans. The first identification of the origin of SARS-CoV was the detection of the virus in some wild animals sold at fairs, such as Himalayan civets (*Paguma larvata*) and a Raccoon dog (*Nyctereutes procynonoides*), in addition to some merchants who had contact with the animals, showed antibodies to the SARS-CoV virus, although they did not manifest the disease [122,123].

Patients infected with SARS-CoV had broad clinical manifestations, the main symptoms of which were fever, myalgia, malaise, and symptoms in the lower respiratory tract, such as cough and dyspnea and, subsequently, severe pneumonia. In addition, gastrointestinal symptoms have been reported in some patients, such as diarrhea. The main routes of transmission described were contamination by infected droplets dispersed in the air by infected patients, which were expelled through coughing or sneezing, as well as personal contact and contact with contaminated surfaces. Its transmission was quickly interrupted by public health measures and, since then, no other SARS-CoV infection has been observed in humans [2,123,124].

3.3. Severe acute respiratory syndrome (SARS) − 2

In December 2019, a new coronavirus was identified as the main cause of pneumonia in Wuhan, in the province of Hubei in China, called 2019-nCoV. Subsequently, the International Committee on Taxonomy of Viruses (ICTV) named this new virus as severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) and the WHO announced a standard format for 2019 Coronavirus disease, called COVID-19. With the spread of the virus to several countries, the pandemic was declared on March 11, 2020 by the WHO [5,115]. Research on the origin of the spread of SARS-CoV-2 suggests that it started in a market in Wuhan, which sold various types of live wild animals. Thus, it was possible to isolate the virus in samples of commercialized products, however, the first confirmed patient had no relationship with the market, corroborating a contradictory etiology [5].

In SARS-CoV-2 infection, symptoms are similar to those of SARS discovered in 2002, such as fever, rhinorrhea, headache, fatigue, and dry cough, may occur. In addition to this condition, patients may also experience diarrhea, anosmia, ageusia and odinogafia, and in more severe cases pneumonia is present. Although approximately 80% of patients have mild symptoms, in severe cases, patients may experience lymphopenia and interstitial pneumonia, with high levels of pro-inflammatory cytokines, which makes it difficult to contour to a healthy state [125,126].

According to studies, symptoms begin to appear after an incubation period of approximately five to seven days of the virus in the human body. The period between the onset of symptoms and worsening, in some cases death, varies from 6 to 41 days, although patients over 70 years of age and / or with compromised immune systems may have a shorter period [127].

The mode of transmission is based on the knowledge of another similar coronavirus, mainly SARS-CoV, in which the main form of transmission is through contaminated respiratory droplets, expelled through coughing or sneezing, as well as direct contact with mucous membranes in the mouth, eyes, and nose, or even contact with infected surfaces followed by contact with mucous membranes. The drop range can be up to two meters and does not remain in the air, however, SARS-CoV-2 is viable in aerosols for up to three hours. It should be noted that the coronavirus can also be transmitted during the virus incubation period, therefore, asymptomatic patients are considered to be potential sources of infection. In addition, SARS-CoV-2 ribonucleic acid (RNA) was detected in blood and stool samples, suggesting that transmission can also occur in the orofecal form, although it is not significant in dissemination [5,128].

The diagnosis of COVID-19 is made from samples of the upper respiratory tract (nasopharyngeal or oropharyngeal) and of the lower respiratory tract (endotracheal tube or bronchoalveolar lavage). The standard method for identifying the virus is by the reverse transcription polymerase chain reaction (RT-PCR) of the SARS-CoV-2 nucleic acid. Roche's commercial SARS-CoV-2 test system (cobas® SARS-CoV-2) has been approved by the US Food and Drug Administration (FDA), this test requires samples of nasopharyngeal or oropharyngeal swabs and its method of analysis is based on the RT-PCR protocol, as it detects the SARS-CoV-2 RNA. In clinical practice, chest radiography or computed tomography CT findings are an important tool for the diagnosis of COVID-19, due to the characteristics of the images, which include bilateral distribution of irregular shadows and ground-glass opacity [126,128].

In addition, mutations are great allies of coronaviruses and the mechanism of genomic revision hinders treatments, including the use of antivirals. This is because the mechanism of action that was initially specific becomes obsolete after a large number of mutations, such as the influenza virus, which requires a new vaccine each year [129]. Thus, the need to study the immune system is visible to understand the mechanism used by the virus and to be able to critically evaluate future therapies.

3.3.1. Infection mechanism and inflammatory responses

In the mid-1960s, the coronavirus family was first identified and then classified into four subfamilies: α (alpha), β (beta), γ (gamma), and δ (delta) - the first two mainly infect mammals and the others usually infect birds. On December 30, 2019, another virus belonging to the β (beta) subfamily, SARS-CoV-2 (currently seven types of coronavirus have been identified) that has single-stranded RNA was identified, analyzed, described, and classified. This is an enveloped positive-sense RNA virus presenting in its structure some main proteins such as the spike (S), envelop (E), membrane (M), and nucleocapsid (N) proteins [130]. SARS-CoV-2 has the ability to recombine pieces of its RNA with others in the coronavirus family, so when these exchanges occur, they lead to new versions of the virus and these are often found in bats, and may end up infecting humans [129].

SARS-CoV-2 is able to be endocitated by the cell when its S protein is anchored to the ACE2 receptor, which is found in the nasal mucosa, bronchi, lung, heart, esophagus, among other organs. Thus, together with the host's proteases, mainly TMPRSS2, the virus is able to invade the cell, generating a structural rearrangement to fuse its viral membrane with the membrane of the host cell. After fusion, the N protein is responsible for replication and becomes the main antigen used for the immune response [5,115]. As a result, both the innate and adaptive immune systems are activated in the viral response [131] and what is being highlighted in research is the different mechanism of SARS-CoV-2 in the direct inhibition of the expression and signaling of IFN-I [132].

Thus, with the virus installed in the host cell, the innate immune response is activated, which recruits neutrophils, macrophages, and other immune cells for the initial attack. However, due to the complexity of the pathogen and the duration of antigen signaling, lymphocyte activation is also necessary. Thus, CD4 + T cells are activated and provide differentiation into specialized T cells (Th1), contributing to increased secretion of pro-inflammatory cytokines, such as interleukin 2 (IL-2), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 1 beta (IL-1β), a granulocyte colony-stimulating factor (G-CSF), interferon gamma-induced protein 10 (IP-10), monocyte chemotactic protein 1 (MCP-1),

macrophage inflammatory protein 1-alpha (MIP-1α), and tumor necrosis factor alpha (TNF-α), to act in the inflammatory process. However, as there is no tightening of the attack, the cycle is replenished, exaggerating the inflammatory markers and generating the cytokine storm, which can cause ARDS, organ failure and, if not bypassed, the patient's death [115,118,133].

In contrast to that found in SARS, patients with COVID-19 may have elevated levels of cytokines secreted by Th2 cells, such as IL-4 and IL-10, which have an anti-inflammatory action, but are not able to inhibit the inflammatory condition [134]. Thus, studies suggest a correlation between the cytokine storm and the severity of the disease; patients in critical stages have the highest levels of pro-inflammatory mediators in relation to moderate and mild patients (critical patients > critically ill patients > common patients) and IL- 10 could be able to reduce the negative effects if significantly concentrated [135].

The virus antigen in the body can activate CD4 + T cells and CD8 + T cells, which play a significant antiviral role in order to return homeostasis and fight the pathogen. After the activation of specific CD8 + T cells by means of the major histocompatibility complex class I (MHC-I), these cells proliferate and differentiate to promote cytotoxic effects. CD4 + T cells can also elicit responses after interacting with the major histocompatibility complex class II (MHC-II) of antigen presenting cells (APCs), participating in immune response processes [5,130]. CD4 + T cells promote the production of specific antibodies against the virus, activating B cells, while CD8 + T cells are cytotoxic and can kill infected viral cells [5,115] (Fig. 1).

In the ongoing process of fighting viral infections, specific and non-specific immune responses work together to produce an efficient response. However, the specific immune response is the key factor in completing the elimination of the virus in the body [5]. In this inflammatory context, the release of extracellular nucleosides and nucleotides triggers the increase of purinergic signaling in the body, which modulates different pro- and anti-inflammatory actions. Thus, it is necessary to understand how the purinergic system affects the immune system and the inflammatory processes in the pulmonary tissue during the course of COVID-19, especially considering that the modulation of these systems components and of inflammatory responses is key for the disease outcome.
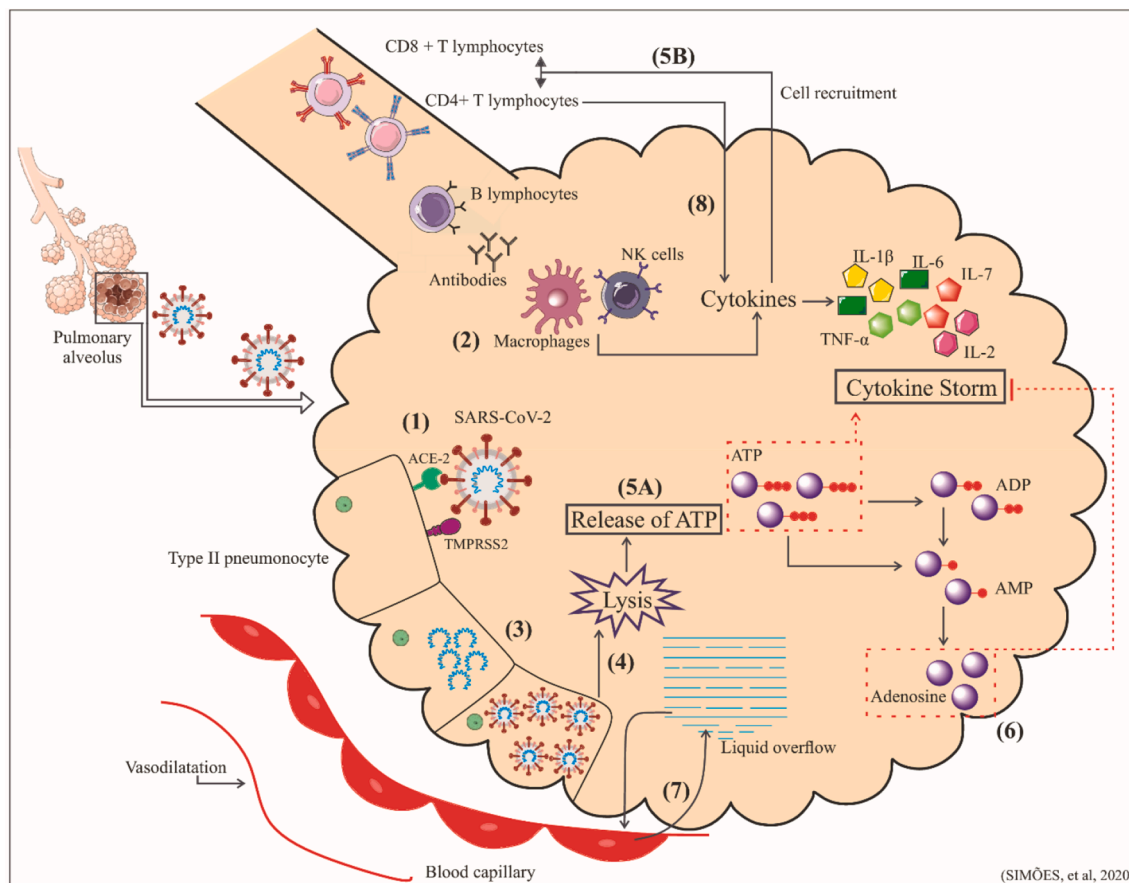

Fig. 1. Representation of the hyperinflammation status caused by the SARS-CoV-2 virus associated with the most serious cases of COVID-19. (1) The SARS-CoV-2 virus can enter the body through the airways and reach the pulmonary alveoli, infecting alveolar cells (type II pneumocytes). In pneumocytes, the virus binds to ACE2 and TMPRSS2 via the surface S protein and inoculates its genetic material into the cell. (2) After the virus enters the body, the innate immunity is activated, thus promoting the activation and recruitment of macrophages and NK cells. to the site of infection. Macrophages promote the release of pro-inflammatory cytokines. (3) Inside the cells, the virus uses all the cellular organelles to replicate and thus make several copies of it. (4) With numerous viral copies in the intracellular compartment, the cell enters a lyses state. (5A) With cell lysis, numerous molecules and damage signals are released, including ATP, which may activate the purinergic system signaling. (5B) Concomitantly, the cytokines released by macrophages trigger the acute inflammatory response, which recruits CD8 + and CD4 + T lymphocytes. (6) The activation of the purinergic signaling results in the formation of a large amount of Ado from the initial ATP released by the cells that underwent lysis. (7) With all this acute inflammatory response, the alveoli are filled with water, thus characterizing pneumonia. Blood vessels dilate and permeability also increases. (8) CD4 + T lymphocytes release more pro-inflammatory cytokines that recruit more CD4 + T lymphocytes. Through this positive feedback, the pulmonary inflammatory response increases, this leads to a cytokine storm. In this scenario of hyperinflammatory response, with pneumonia and hypoxia, it is evident that the inflammation has shifted from physiological to harmful and pathological, representing the most severe cases of COVID-19.

4. Purinergic signaling and inflammatory responses in SARS-CoV-2 infection and other pathologies

From the above, the regulatory and essential role of the purinergic system in the inflammatory process is understandable [136], so it is valid to relate its therapeutic potential in the context of the 21st century pandemic. Purinergic receptors are generally encountered in most immune cells [137,138], thus, these cells are able to release ATP to the extracellular medium, which acts by modulating the receptors expressed on cell membranes via autocrine or paracrine signaling. B lymphocytes, for example, undergo increased cell proliferation in response to ATP [138], besides, dendritic cells, monocytes, NK cells, macrophages, and eosinophils, also have their migration regulated by this nucleotide [139]. Thus, besides immune cells, surrounding tissue cells may also release ATP, widely extending the cellular intercommunication network. In this regard, purinergic signaling becomes a major checkpoint in the activation and control of the functional responses of immune cells.

In this perspective, the cytokine storm generated by SARS-CoV-2 is capable of releasing pro-inflammatory cytokines acting as signals at purinoreceptors. After viral entry and replication in the cell, the process of lysis and release of ATP is responsible for the activation of extracellular receptors such as the P2X7 expressed on immune cells. For instance, P2X7 stimulation in monocytes and neutrophils leads to the activation of the canonical NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome pathway, prompting an exaggerated release of IL-1β, a major pro-inflammatory cytokine during the cytokine storm [140].

The neutrophil attack mechanism consists of the production and release of reactive oxygen species (ROS), which increases the chemotaxis of the site, resulting in a greater recruitment of immune cells and consequent inflammation. This situation can be overcome by extracellular Ado in the activation of A2A receptors, reducing the immune activation. Furthermore, Ahmadi's study demonstrated, by analyzing the expression of CD39 and CD73 in CD4 + and CD8 + T cells, as well as in natural killer cells from patients with COVID-19, a reduction in enzymatic activity. Thus, the absence of CD73 on CD8 + and NKT T cells allows greater secretion of cytokines, such as TNF-α and interferon gamma (IFN-γ) [141].

Thus, the purinergic role in the activation of several types of immune cells is conspicuous. ATP, for example, is released by panexin channels with the activation of T lymphocytes and participates in the immune synapse, enabling the influx of calcium and cellular activation [142]. Neutrophils, on the other hand, secrete ATP in response to chemotactic mediators, as well as regulate the chemotaxis process by activating P2 receptors expressed on their membrane [143]. In addition, immune cells share positive or negative feedback responses in order to regulate their actions. Moreover, molecules such as ATP and Ado, besides regulating immune cells functions, also serve as signaling mechanisms to surrounding cells, especially to AECs, indicating that the purinergic system plays a fundamental role in disease progression when considering the pulmonary tissue [144].

With the onset of infection, the SARS-CoV-2 virus attack mechanism impairs the rapid increase in IFN-I, which characterizes the "initial alarm" to the innate immune system, enabling the virus to replicate [131]. With this relative delay in IFN-I signaling and the rapid kinetics of coronavirus replication, an inadequate inflammatory response is induced, with extensive vascular leakage, specific T cell responses to impaired viruses and consequent pulmonary immunopathology [145]. Thus, studies describe that the concentration of ATP is closely associated with this mechanism, which can neutralize this inhibition, facilitating the secretion of IFN-I through the P38 / JNK / ATF-2 signaling pathway [63]. However, the scenario of the cytokine storm in the pulmonary tissue is subsequent to the initial replication of the virus, therefore, with the inflammatory process already establish and uncontrolled, the action of the pro-inflammatory ATP becomes harmful, requiring

immunological suppression triggered by the action of Ado [146].

The purinergic system, due to its immunomodulatory capability, may be a common feature in acute and chronic lung diseases, including ARDS, asthma, COPD, and idiopathic pulmonary fibrosis (IPF), hence providing therapeutic potential; however, the role of signaling molecules, such as nucleotides and nucleosides in these pathological conditions is still largely unknown. In relation to ATP, in the intracellular environment, this nucleotide is the end product of reactions such as photophosphorylation, aerobic and anaerobic cell respiration (fermentation); ATP is a core molecule in energy homeostasis, as well as in endergonic biosynthetic processes, presenting a central role as an energy donor and supporting cell survival, proliferation, and motility. Nevertheless, once in the extracellular milieu, ATP has a signaling role and acts in cell proliferation, mitogenesis, and differentiation [147].

In addition, high concentrations of ATP in the outer cellular space, due to cell death and lysis, can function as danger-associated molecular patterns (DAMPs), eliciting inflammatory responses through the binding of this molecule to P2X7 receptors, expressed on the surface of immune cells, triggering an internal signaling cascade with the assembly of the NLRP3 inflammasome and posterior IL-1β secretion. The cellular signaling cascades activated by the ATP/P2X7 axis have been linked to the onset of neurodegenerative morbidities and also to inflammatory-associated conditions, thus deserving further investigations to elucidate the underlying mechanisms [148-150].

On the other hand, although Ado has been reported extracellularly in situations of hypoxia, inflammation, and trauma [20,91], this molecule has been acknowledged to decreasing heart rate and increasing coronary blood flow [151], besides being used as coronary vasodilator [152]. COVID-19 patients may develop hypoxia and suffer from systemic and generalized inflammation [20,153], therefore, the study of Ado seems promising in this pathology, possibly preventing and mitigating pulmonary inflammations, whether acute or chronic. Likewise, the amount of cytokines released by individuals infected with SARS-CoV-2 is capable of generating an increase in extracellular ATP and, considering that in high concentrations it can cause cytotoxic effects [22], studying the actions of this nucleotide is necessary to reduce or prevent the cytokine storm elicited by the viral infection.

The extracellular ATP, released upon cell injury or lysis, will be promptly metabolized into ADP and then AMP by NTPDase, followed by hydrolysis of AMP into Ado by CD73. Ado, by the binding to its four subtypes of P1 receptors, may signal pro- or anti-inflammatory effects. Subsequently, this molecule may be further deaminated into inosine by ADA, intracellular transport by concentrated or equilibrative nucleoside transporters (CNTs and ENTs, respectively) or used to regenerate AMP by phosphorylation through the adenosine kinase (AK) pathway [33].

In the events of inflammatory and pulmonary hypoxia, there is an increase in the extracellular levels of ATP, ADP, and Ado [154,155], therefore, these molecules are central to lung injuries due to their effects on remodeling, repair, and inflammation processes. However, these actions can be protective or destructive to the tissue, since Ado may exert an anti-inflammatory and protective action when activating A2A and A2B receptors during acute events. However, in chronic situations, Ado may function in an opposite manner due to excessive signaling of this nucleoside, promoting pro-inflammatory and destructive effects [154].

Furthermore, in a scenario of generalized inflammation, that are more likely to occur in severe COVID-19 cases, there may be an unregulated activation and excessive recruitment of fibroblasts, airway epithelial cells (AECs), macrophages, neutrophils, eosinophils, and myofibroblasts. In addition to the hypoxia scenario and hypoxia-inducible factors (HIFs), there is also a signaling for increased extracellular Ado via transcriptional regulation of Ado metabolizers and receptor genes [156-161].

In this way, the established inflammatory process generated by the body induces the permanence of a situation of defense and constant release of more pro-inflammatory mediators such as TNF-α and IL-6 that

potentiate excessive angiogenesis, the remodeling of the pulmonary tissue, including the extracellular matrix and airway epithelial cells [93,162], and also systemic inflammation [162-164]. Therefore, it is necessary to understand how the pathophysiology of acute and chronic lung diseases is associated with the purinergic system.

Purinergic signaling in the cytokine storm and thrombotic process of COVID-19

Complications caused by COVID-19, such as ARDS and involvement of other organs, are characterized by exaggerated immunoinflammatory responses. Thus, the high release of pro-inflammatory factors in the bloodstream and systemic inflammation is called cytokine storm and is present in severe cases of COVID-19 [140,165]. In view of this, cytokine storm is determined by elevated levels of interleukins such as interleukin IL-1β, IL-6, IL-2, IL-7, G-CSF, IFN-γ, TNF-α and various other cytokines and pro-inflammatory chemokines [135,140].

The uncontrolled infection mechanism is closely related to the body's immune response to infection. With the entry of the SARS-CoV-2 virus through the respiratory tract, infection occurs mainly in lung epithelial cells. However, macrophages and dendritic cells can also be infected, leading to hyperinflammation, and consequently weakening the immune system [140]. According to Pacheco and Faria [166], there are associated changes in the immune system and purinergic signaling, since, in viral infections, cells of the innate immune system recognize the foreign body and release ATP due to cellular stress.

From this, ATP promotes a pro-inflammatory and chemotactic response to macrophages and neutrophils, being considered a DAMP. Added to this, the release of the nucleotide by the injured cells in the extracellular environment act as autocrine and paracrine signaling to nearby cells and tissues. With the increase in concentration levels, there is activation of purinergic receptors such as P2X7, which is strongly expressed in T cells, macrophages and neutrophils, and has the function of activating the immune system and generating an inflammatory environment. From this, the activation of the P2X7 receptor leads to an increase in the levels of NLRP3 inflammasome, which is involved in viral infections, after its activation it changes to a multiprotein aggregate composed of several NLRP3 molecules, resulting in exacerbated production of IL-1β , according to Ribeiro, et al. [167] this inflammasome acts as a trigger in the cytokine storm, induced by the P2X7 receptor [140,167]. Furthermore, after activation of the NLRP3 inflammasome, ATP is also released into the extracellular environment, which causes a positive feedback cycle [167]. Other receptors such as P2Y6R, when expressed in excess, generate inflammatory responses with high levels of cytokines IL-1β, IL-6 and TNF-α, triggering damage to the pulmonary epithelium and endothelium. As well, the P2Y2R receptors are also involved in the exacerbated inflammatory response, contributing to the development of cytokine storm [166].

In view of this, the purinergic system represents an important area of study for the development of substances that block nucleotide and nucleoside receptors. Proving to be a possible therapeutic target in COVID-19, as they would reduce the purinergic signaling cascade and consequently the pro-inflammatory effects of the cytokine cascade associated with lung and other organ damage caused by the virus [34,140,167-169].

Another aspect to be highlighted in a viral infection is blood coagulation, which undergoes changes in the clinical picture of COVID-19 and is closely associated with purinergic signaling [165]. Findings shows that a significant number of patients diagnosed with COVID-19 have thromboembolism and other damage from disordered blood coagulation, especially in severe cases of the disease [170]. With the elevation of ATP levels and macrophage activation, there is a modulation of leukocytes and platelets by the activation of purinergic receptors [171]. Furthermore, acting directly on platelets, CD39 ectonucleotidase through a degradation process transforms ATP into ADP, signals platelet aggregation by activating P2Y12R [168]. However, in addition to these platelet aggregation factors, it was possible to observe in patients with COVID-19, the exacerbated formation of fibrin and consequently an increase in D-dimers, and also an excessive release of IL-6, causing an increase in fibrinogen levels [170,172].

In addition to an inflamed environment with the manifestation of immune system cells, ATP release and conversion to ADP, activation of receptors involved in the inflammatory process, there is also a release of neutrophil extracellular traps (NET) that activate platelet factor XII, contributing once again to platelet aggregation, causing harm to patients [170]. Thus, the thrombotic process involved with COVID-19 has two main points involved, the aggregation of intra-alveolar fibrin and the formation of clots through the activation of platelets through the activation of P2Y12 factor XII receptors, which can generate arterial thrombosis, embolism pulmonary, deep vein thrombosis and thrombotic microangiopathy, and these are associated with severe cases and deaths [170,173].

5. Therapeutic potential of purinergic receptors in COVID-19

5.1. P1 receptors

The therapeutic capacity of purinergic receptors in the case of SARS-CoV-2 infection and associated respiratory symptoms is thought to be linked to immunomodulatory functions mediated by purinergic signaling pathways. By way of example, in acute lung injuries, A2A and A2B receptors can play anti-inflammatory and protective functions [93,174,175]. These findings are consistent with the protective actions of extracellular adenosine in acute pulmonary inflammation. Thus, there is an attenuation of inflammation, increased tissue tolerance to ischemia, as well as an improvement in the reestablishment of normal oxygenation [41,176-178].

In this sense, the use of adenosine or NECA (5′-N-ethyl-carboxamidoadenosine - non-selective AR agonist), in a murine model of lung inflammation induced by intratracheal LPS, showed an improvement in the barrier function of the lung tissue, as well as a reduction in lung inflammation. Inflammation evidenced by reduced neutrophil and cytokine infiltration. In addition, A2AR activation was able to attenuate pulmonary inflammation and edema, improving gas exchange and physiological respiratory function [176,179]. Furthermore, the activation of the A3 receptor by the selective agonist was able to reduce the inflammatory picture and pulmonary edema, as well as the levels of cytokines, chemotaxis and activation of neutrophils [180].

Research also shows that A2BR deletion or blockade exacerbated inflammation and edema [174] and the use of its selective agonist BAY60-6583 is able to decrease AECs apoptosis in pulmonary inflammation [181]. Thus, a decrease in edema and an improvement in gas exchange can be observed due to ENT-dependent A2B activation [182] and, in another study, it provided pulmonary protection [183]. This is because, due to induced lung injury and arterial injury, macrophage activity increases the regulation of this receptor [184], as well as its expression reduces TNF-α levels.

Activation of other adenosine receptors was also able to modulate lung inflammation after ischemia–reperfusion (IR) injury, as well as influenza infection. Receptors such as A3A, which are present in lung tissue and inflammatory cells, can reduce lung inflammation and edema. In addition, research in mice observed a reduction in cytokine levels and chemotaxis responsible for the recruitment and activation of a greater number of neutrophils [180].

However, although activation of A2AAR, A2BAR, and A3AR mediate protective effects in acute lung injury, signaling through A1AR is harmful and appears to be harmful. Studies indicate that the activation of A1AR adenosine promotes pulmonary recruitment of innate immune cells and the progression of lung injury. Thus, the A1AR antagonist acted to reduce lung damage in mice infected with influenza [185].

Another interesting fact that can modulate the concentration of signaling molecules is the expression of ectonucleotidases CD39 and CD73. In cases of acute pulmonary inflammation, high levels of CD73 on lymphoid cells and CD73 on myeloid cells are observed. Furthermore,

accelerated activity was observed in the hydrolysis of ATP to adenosine by TCD4 + cells, added to the significant expression of A2AR in helper T cells. Thus, since T cells play an important role in the tissue repair process after acute lung inflammation, the increase in adenosine and A2B upregulation is closely associated with a repair scenario [186]. The following table includes P1 receptors and their actions in COVID-19 pulmonary pathologies (Table 1).

5.2. P2 receptors

In an inflammatory setting, P2X and P2Y receptors appear to be linked to cell necrosis [187], and their downregulation with protection against diseases such as asthma, vascular inflammation, and graft-versus-host disease (GvHD) [188-190]. Its antagonists, in turn, act to inhibit this inflammatory condition in inflammatory diseases [158,187,191]. Thus, P2X7 blockade may be related to tissue protective effects in these cases [137]. Studies that induced lung inflammation by high tidal volume mechanical ventilation demonstrated that P2Y is

active in increasing inflammatory conditions [192]. In addition, P2X7 is related to the production and secretion of surfactants and high levels of extracellular ATP can alter the function of receptors and decrease the amount of surfactant, triggering edema and acute injury [193-196] (Table 2).

Dagvador et al. reported that, in an inflammatory lung environment induced by LPS, alveolar macrophages (AM) underwent necrosis due to activation of the P2X7 receptor. These necrotic AMs release pro-interleukin 1α (IL-1α), responsible for activating endothelial cells and increasing vascular permeability, increasing local inflammation. Thus, P2X7R deficiency or blockade in this study attenuated AM necrosis and reduced pro-IL-1α secretion [197].

In addition, in pulmonary inflammation caused by mechanical ventilation, a considerable increase in the concentration of ATP in the alveolar bronchial lavage fluid (BALF) was observed. One of the ways ATP induces inflammation is by activating the P2Y receptor, as the use of its specific antagonism was able to partially attenuate the inflammatory response, suggesting an ATP-P2Y association in ventilator-induced lung inflammation [192]. An example of receptors in this family is the $P2Y_6R$ which, induced in endothelial cells after exposure to LPS, resulted in increased vascular inflammation [188].

Furthermore, in viral infections, ATP release increases the production of interferon type 1, which limits virus replication in infected cells [63]. Thus, the protective role of NLRP3 inflammasomes in the early course of the infection is understood. However, with uncontrolled infection and exacerbation of inflammation, the action becomes detrimental due to immunopathogenic effects [198]. A fact demonstrated in influenza A in mice, but that is closely associated with cases of COVID-

Table 1

Actions of P1 receptors in the inflammatory process of pulmonary pathologies.

Disease/ Condition/ Experimental model	Action mechanism/ Outcome	Receptor(s)	Reference(s)
Acute lung injury	Anti-inflammatory and tissue protector	A2A A2B	[93,174,175]
Acute lung injury	Attenuate inflammation and edema	A2A	[176,179]
Lung injury	Protective role	A2B	[174]
Pulmonary inflammation	Mitigation of pulmonary inflammation and edema	A2B deletion in AECs	[181]
Pulmonary inflammation	Decrease in AECs apoptosis	A2B (using a selective agonist (BAY60-6583)	[163]
Pulmonary injury in AECs	Decrease in AECs apoptosis	A2B activation	[183,184]
Lung injury induced by traumatic hemorrhagic shock	Lung protection	A2B activation	[162]
Arterial injury in mice	Increased A2B regulation by macrophages	A2B	[183]
Arterial injury in mice	Decreased TNF-α production	A2B	[184]
Lung tissue and inflammatory cells	Decreases inflammation, pulmonary edema, cytokine levels, chemotaxis, and neutrophil activation	A3 activation with a selective agonist	[180]
Acute lung inflammation after influenza infection	Pulmonary protection	A2A A2B A3	[180]
Acute inflammation	Edema and inflammation	A1	[185]
Pulmonary inflammation	Pulmonary recruitment of innate immune cells and progression of lung injury	A1	[186]
Influenza infected mice	Mitigates lung injury	A1 antagonism	[185]
Acute lung inflammation	Decrease in pulmonary edema and improvement repair	A2A in helper T cells	[185]
Acute lung inflammation	Repair of pulmonary tissue damage	A2B in lung cells	[186]

Table 2

Actions of P2 receptors in the inflammatory process of pulmonary pathologies.

Disease/Condition/ Experimental model	Action mechanism/ Outcome	Receptor(s)	Reference(s)
Pulmonary inflammation	Cell necrosis	P2X e P2Y	[187]
Asthma, vascular inflammation, and Graft-versus-host disease (GvHD)	Protection against these disease conditions	Deletion of P2 receptors	[188-190]
Inflammatory bowel diseases, lung inflammation, and ischemia–reperfusion injury	Inflammation inhibition	P2R antagonist	[158,187,191]
Inflammatory Diseases	Protective effects to tissues	P2X7 antagonist	[137]
Mechanical ventilation-induced lung inflammation	Pro-inflammatory effects	P2Y	[192]
P2X7 receptor activation in alveolar macrophages (AMs)	Necrosis	P2X7	[197]
Murine hyperoxia model of acute lung injury	Tissue protective effects	P2X7	[197]
P2X7 receptor downregulation	Hyperoxic-induced lung inflammation	P2X7	[197]
Pulmonary inflammation caused by high tidal volume mechanical ventilation	Pulmonary inflammation	P2Y	[188,192]
Pulmonary inflammation	Reduction of lung inflammation	P2X7 antagonism	[148,189,199-201]
Production and secretion of surfactants	High levels of extracellular ATP, decreased surfactant, edema, and acute injury. P2X7 receptor in the maintenance of surfactants	P2X7	[192-196]

19.

Thus, the involvement of P2X7R in inducing harmful hyper-inflammation is remarkable, as well as the therapeutic potential of its inhibition to limit tissue damage. Studies indicate that its pharmacological inhibition improved influenza A pneumonia in mice [199], in addition to being related to pulmonary tuberculosis [148] and ARDS [189,200,201].

In addition, to further support the close relationship between the treatment capacity of P2 and COVID-19 receptors, a recent report provided information on the involvement of the P2X7 receptor and the neuropathological processes triggered by SARS-CoV-2 infection. From this perspective, the central role of the P2X7 receptor, and the subsequent inflammatory processes stimulated by its hyperactivation, identified in neurodegenerative and psychiatric morbidities, could shed some light on possible pharmacological interventions to help with neurological and pulmonary complications in COVID-19 patients [167].

6. Future therapeutic proposals

Therefore, the modulation of components of the purinergic system seems promising to reduce the inflammatory condition in the pulmonary tissue in the face of uncontrolled and general involvement by the cytokine storm. In relation to P2 receptors, the administration of P2X7 receptor antagonists may be able to protect against respiratory and inflammatory diseases, possibly due to the reducing action in the

activation of the NLRP3 inflammasome and release of IL-1β, added to a protection against edema and acute injury by the maintenance of alveolar surfactants. Ado receptors, on the other hand, must be signaled and activated, either by the administration of agonists or by increasing the concentration of the nucleoside, due to its anti-inflammatory and protective role. Thus, P2X7R blockade and A2AR and A2BR activation are highlighted as possible therapeutic targets in order to reduce edema and lung damage. In addition, the use of selective A1 agonists is reported to be able to reduce levels of cytokines, chemotaxis and immune system responses, reducing inflammation and pulmonary edemA (Fig. 2).

7. Conclusions

In the process of viral infection, the involvement of the immune system is fundamental in the fight and recovery, however, different levels of inflammatory responses may occur, varying from one individual to another. With an accelerated replication, SARS-CoV-2 quickly activates cells of the immune system, which secrete pro-inflammatory cytokines which, if exaggerated and uncontrolled, can cause the cytokine storm. This, in turn, triggers an increase in the concentration of ATP in the extracellular medium, which is able to increase purinergic signaling through its binding to the P2X7 purinoreceptor, stimulating the release of more pro-inflammatory cytokines. The most severe cases of COVID-19 that present pneumonia, hypoxia, and SARS also have a hyperinflammatory response, so, it is evident in these cases that the
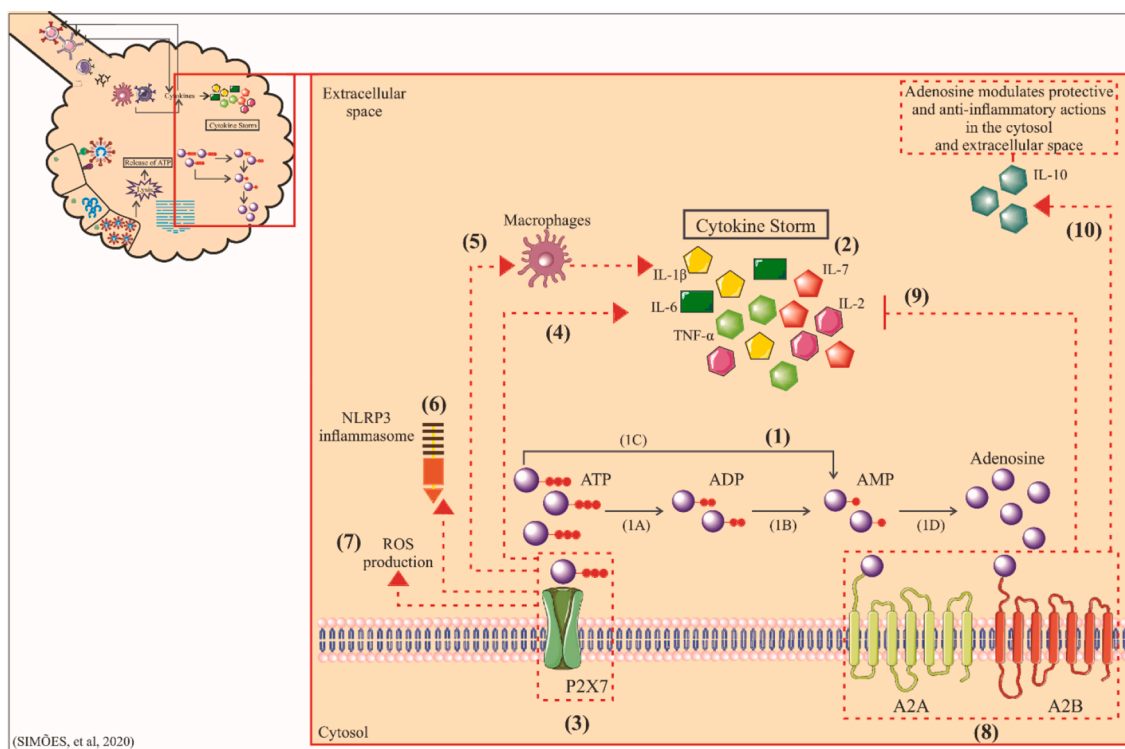

Fig. 2. Representation of the correlation between the cytokine storm caused by the SARS-CoV-2 virus and the hypothesis of the involvement of the biomolecules of the purinergic signaling system with the disease. (1) The increase in extracellular ATP concentrations activates a hydrolysis cascade, sequentially degrading this nucleotide into ADP and AMP by the E-NTPDase enzyme (steps 1A and 1B), or directly into AMP by the E-NPP enzyme (step 1C). AMP is further hydrolyzed to Ado by the activity of the E-5'-NT enzyme (step 1D), generating large extracellular amounts of Ado. (2) The cytokine storm comprises an inflammatory event caused by the SARS-CoV-2 virus, with the release of IL-1β, IL-2, IL-6, IL-7, and TNF-α cytokines. (3) Extracellular ATP binds to the P2X7 purinoreceptor. (4) P2X7 is associated with the direct release of pro-inflammatory cytokines promoting an increase in the cytokine storm.. (5) The P2X7 purinoreceptor can act by stimulating macrophages, which, in turn, induces the release of pro-inflammatory cytokines. (6) P2X7 also stimulates the activation of the NLRP3 inflammasome. (7) This purinoreceptor may also promote the increase in the production of ROS and, consequently, increase the pro-inflammatory state. (8) The hydrolysis of purinergic biomolecules promotes the formation of large amounts of Ado, which binds to the A2A and A2B purinergic receptors. (9) The agonist action of Ado on A2A and A2B receptors inhibits the secretion of pro-inflammatory cytokines, decreasing the cytokine storm and, consequently, restoring homeostasis. (10) The stimulation of these purinoreceptors sensitive to Ado promotes the secretion of anti-inflammatory cytokines, mainly IL-10, which modulate protective and anti-inflammatory actions in the cytosol and in the extracellular environment. It is assumed that the components of the purinergic system, mainly ATP and Ado, and the P2X7, A2A, and A2B purinoreceptors can correlate with the prognosis of the infection caused by SARS-CoV-2, comprising possible pharmacological targets for further study.

inflammation is no longer physiological and has become pathological. Thus, Ado can bind to A2A and A2B purinoreceptors, inhibiting the release of pro-inflammatory cytokines and stimulating the secretion of anti-inflammatory cytokines, such as IL-10, modulating the protective and anti-inflammatory actions, both in the extracellular environment and in the cytosol. Therefore, this study illustrates some of the inflammatory and molecular mechanisms underlying SARS-CoV-2 infection in the pulmonary tissue and assumes that components of the purinergic system, such as ATP and Ado molecules as well as P2X7, A2A, and A2B purinoreceptors, may improve the prognosis of COVID-19 severe cases and may be targets for possible therapies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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